

EXPERIMENTAL STUDIES OF THE NASOPHARYNGEAL
SECRETIONS FROM INFLUENZA PATIENTS.

I. TRANSMISSION EXPERIMENTS WITH NASOPHARYNGEAL WASHINGS.

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PLATES 5 TO 7.

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INTRODUCTION.

In planning the present experiments we had in mind the possible presence in the nasopharynx of persons suffering from acute epidemic influenza of some agent the effects of which might be noted in animals. In considering the criteria of activity of this agent we thought, first, of the well known phenomenon in man of leucocytic depression, involving especially the mononuclear cells, during the acute influenzal attack, and next, of changes of a more or less pronounced but possibly transient character, arising in the lungs, which might conceivably predispose to the severe pneumonias that often accompany as a secondary or concurrent infection the influenzal attack.

Furthermore, this study was made during the course of over 1½ years in three successive periods. The first period coincided with the epidemic wave of 1918-19. During this period cases of acute uncomplicated influenza and individuals who had never been affected were studied. The second period embraced the late autumn of 1919, during which influenza did not prevail in New York in epidemic form. During this interepidemic period normal individuals were studied as controls. The third period, the winter of 1920, saw a return of the epidemic. At this time additional cases of the disease were available for investigation. By proceeding in this manner we hoped to check the results for each period against the others. As the sequel will show, we believe that we succeeded in this undertaking, with the consequence that we are enabled to present our findings with perhaps a degree of confidence not otherwise appropriate.

Materials.

The outstanding difficulty in the choice of materials to be employed arose from the necessity of selecting cases of undoubted acute influenza, on the one hand, and of perfectly healthy individuals, who had never suffered from the disease, on the other. In the end the second requirement was more easily fulfilled, as the circumstances of the undertaking admitted of leisurely and painstaking choice of subjects. With uncomplicated influenza, however, the individuals had to be chosen at once, since the epidemic wave of the disease is notably brief, being prolonged chiefly by secondary respiratory infections.

The criteria which were used as guides in the selection of cases of pure influenza were abrupt onset with chilliness, fever, prostration, headache, and muscular pains, especially in the back and limbs. Among the early symptoms were flush and suffusion of the face, injection of the conjunctivæ, soreness of the throat, and harsh, unproductive cough. In the early stages no physical signs were detected in the chest, gastrointestinal symptoms were inconspicuous, and disturbances referable to other internal organs were not complained of or detected by physical examination.

These symptoms, although striking, were rarely such as could be measured accurately. However, there was one sign that had a quantitative value; namely, the leucocytic picture. Uncomplicated influenza shows a pronounced leucopenia affecting the absolute number of mononuclear cells, chiefly of the lymphocytic variety.¹ This is persistent and even resists at times secondary infectious processes, *e.g.* pneumonia, in which leucocytosis is the rule. As will appear below, great reliance was placed on this quantitative sign in the present experimental studies.

The symptoms and effects endured for from 1 to 3 days, when convalescence, initiated by a lytic fall of temperature, set in, and recovery promptly followed.

¹ The term mononuclears as employed by us includes the leucocytes of the lymphocytic and large mononuclear varieties which have a single homogeneous nucleus. Any indentation of the outline of the nucleus placed the cell in the transitional class, to be counted with the polymorphonuclear cells. Of the varieties of mononuclear cells, the small cells, or lymphocytes, were especially involved in the leucopenia.

Saline washings from the nose and throat were employed. These materials were secured from eight cases of influenza within the first 36 hours of the disease, and from twelve cases at later stages, including the convalescence or the period of postinfluenzal pneumonia. In addition, fourteen individuals who had not been affected were tested during the epidemic or interepidemic period.

Choice of Animal.

In earlier experiments, having in mind a filterable microorganism or virus, we employed *rhesus* monkeys. But this species of animal was found to be unsatisfactory. Monkeys are at best scarce in this country and frequently suffer from pulmonary lesions of a tubercular or other type; the experiments required animals more readily available and free from respiratory affections of any nature. The rabbit was therefore chosen.

EXPERIMENTAL.

Full grown rabbits were used for inoculation, and no rabbit suffering from snuffles or any detectable disease was employed. All animals were subjected to preliminary blood-counting, weighing, and temperature-taking, and any showing variations beyond the average were rejected.² These observations were made on 3 to 7 successive days previous to inoculation. The blood counts were carefully controlled; separate apparatus were used for each animal, which was examined throughout the course of observation by the same technician. Particular stress was laid on the total number of leucocytes and the relative and absolute numbers of polymorphonuclear and mononuclear cells.¹ With regard to temperature, it should be emphasized that in these animals the temperature in itself is no indication at times of the extent or absence of pathological involvement but should be interpreted only in conjunction with other findings.

² For assistance in this and other work we acknowledge our indebtedness to Captain Frank Hornaday, Medical Corps, U. S. Army, and to the Army Laboratory Technicians Miss Mary Jardine, Miss Clara M. McKee, and Miss Anne Webb.

Mode of Inoculation.—The inoculations were made directly into the lungs by means of the intratracheal catheter—a method slightly modified from that employed by Lamar and Meltzer³—or by tracheotomy.⁴

The first method, having the advantage of rapidity of operation, consisted in the insertion intratracheally by way of the mouth of a French silk catheter, size 9. An electric otoscope set in the mouth of the animal served as a gag as well as a guide for directing the catheter. The catheter was curved at 90° $\frac{1}{2}$ inch from the tip. In inserting one should avoid slipping over the larynx into the esophagus, thereby contaminating the catheter.

Although tracheotomy requires more time, it is the preferable procedure because contamination by mouth bacteria is avoided. An incision is made directly over the trachea, and a needle, size 19, bent at a right angle, is inserted therein.

Materials Inoculated.—The materials employed for inoculation consisted of (a) unfiltered nasopharyngeal washings,⁵ (b) filtered washings, (c) lung tissue suspensions,⁶ filtered and unfiltered, from previously inoculated rabbits, (d) similar lung tissue preserved in sterile 50 per cent glycerol, (e) bacteria and culture materials, and (f) control materials. The usual dose for a 2.5 to 3 kilo rabbit was 3 cc. of these materials.

Unfiltered nasal washings were employed in the expectation that they could be purified, or rather deprived of their ordinary bacteria by successive animal passages. It was believed that if this could be accomplished there would be a better chance of preserving and possibly of causing the multiplication of some other variety of microorgan-

³ Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.

⁴ With either method light ether anesthesia was given.

⁵ Nasopharyngeal washings were obtained as follows: The patient's mouth was rinsed with warm saline solution. Then each nasal cavity was washed with 25 to 35 cc. of saline solution, the washings being returned by way of the mouth and collected in a sterile container. The entire fluid was shaken with glass beads in a mechanical shaker for 15 minutes at high speed, or until a homogeneous mixture resulted.

⁶ Lung tissue was prepared for inoculation as follows: The selected portion of the lungs was chopped and then ground with sterile, fine, white sand, and a proportional amount of saline solution was added in the ratio of 20 cc. of saline solution to the two entire lungs. The suspension was then centrifuged at low speed and the clearer supernatant fluid employed for inoculation.

ism, more resistant and virulent perhaps, which would give to the washings from cases of uncomplicated influenza a quality lacking in others. If was, of course, realized that not in every instance could this favorable outcome be looked for. Now and then it was to be expected that a virulent pneumococcus or streptococcus would set up a pneumonia to which the animal would succumb. But if the ordinary bacteria could be suppressed by animal passages in a few instances and something survive which produced definite changes in the tissues of the rabbits—the blood and lungs, for example—the washings from cases of influenza might thus be distinguished in their effects from the washings of another origin. In this way the operation of a pathogenic agent is to be deduced, although it might not be possible to determine certainly that this agent is the inciting microbic agent of influenza. However, if a certain correspondence in tissue and other effects can be shown to exist between the individual suffering from influenza and the rabbit inoculated with materials originally derived from influenza cases and free from all ordinary bacteria, an idea as to the probable nature of the pathogenic agent is gained which encourages further investigation along the indicated lines.

Inoculation of Unfiltered Washings.

There were inoculated into the lungs of rabbits the unfiltered nasopharyngeal secretions derived from five cases of uncomplicated influenza during the first epidemic, and three during the second, in the first 36 hours of the disease, and from eleven cases during the first epidemic and one during the second in the later stages of the affection. The following effects were induced by the materials from seven of the eight early cases, but not by any from the twelve cases examined after 36 hours.

Clinical Effects.—From 24 to 48 hours after inoculation fever developed, associated with the ordinary signs of indisposition in a rabbit, such as listlessness and ruffled hair, and conjunctivitis. The striking feature, however, was the definite and often marked leucopenia resulting from depression of the mononuclear cells, as shown in Text-fig. 1, *a*, *b*, and *c*. If the condition was allowed to run its natural course, these symptoms endured for about 3 days, the animal then returning to

normal. If the rabbit was killed—for in the absence of infection by ordinary bacteria none died—an unusual pathological picture was revealed.

Pathological Effects.—The respiratory organs were affected to the exclusion of all others. No pleuritis or exudate in the pleural cavity was evident. The lungs were voluminous as a result of edema and emphysema and had a mottled hemorrhagic appearance. The hemorrhages on the surface, beneath the pleura, were diffuse or discrete, occupying areas a few millimeters in extent or covering a large part of a lobe. In addition, minute petechiæ were seen scattered over the entire surface. On section of the lungs the cut surface revealed a hemorrhagic edema; it dripped a blood-stained, frothy fluid. The hemorrhages again were either diffuse and large, or discrete and small, in the latter instance being numerous.

On microscopic section carried through various parts of the lungs the lesions were found to consist (*a*) of hemorrhagic foci, and (*b*) of edema and emphysema. The hemorrhages varied in size in accordance with the observed macroscopic appearance, some being microscopic in nature. The edema was more extensive than the hemorrhages and involved alveoli and interalveolar strands of tissue. The alveoli contained coagulated serum or red corpuscles, mononuclear cells, and also at times polymorphonuclear cells of eosinophilic type and desquamated epithelial cells. The interalveolar strands were infiltrated with mononuclear cells and large cells the foreign nature of which was not always clear. Fibrin was sometimes present in small amounts. The bronchi, also, were at times filled with erythrocytes, exfoliated and degenerated epithelia, and leucocytes. The capillaries were distended with blood.

No ordinary bacteria were seen in impression films of the lung tissue or in sections stained by Gram's or MacCallum's⁷ method, or in aerobic or anaerobic cultures of the tissue.

The two following protocols are presented in order to show the clinical and pathological effects, regarded as typical, which arise independently of the presence of ordinary bacteria of any demonstrable kind.

⁷ MacCallum, W. G., The pathology of the pneumonia in the United States Army camps during the winter of 1917-18, Monograph of The Rockefeller Institute for Medical Research, No. 10, New York, 1919, 47.

PROTOCOL 1.

Patient 8.—M. T., adult female. Feb. 9, 1919. Onset sudden with chills and fever. Feb. 10. Photophobia; prostration; muscular pains in the back and extremities; mild unproductive cough. Temperature 37.8°C.; pulse 80. Leucocytes 8,725, of which 1,832 were mononuclears. Physical examination showed lungs to be clear; pharynx red and congested; conjunctivæ injected. No other organs affected. Cultivation of sputum and nasopharyngeal washings yielded *Pneumococcus* Type IV; no Pfeiffer bacilli. The fever persisted for 48 hours and was followed by an uneventful recovery.

Animal Inoculation.—The unfiltered nasopharyngeal washings obtained 20 hours after the onset of the symptoms were inoculated intratracheally into Rabbit A.⁸

First Passage. Rabbit A.—Before injection the normal blood counts showed the following results: Feb. 8, 1919. Leucocytes 13,050, of which there were 6,525 each of polymorphonuclears and mononuclears. Feb. 10. Prior to inoculation, leucocytes 14,425, of which 7,357 were polymorphonuclears and 7,068 mononuclears. Temperature 39.5°C. Injected intratracheally with 3 cc. of the nasopharyngeal washings from Case 8. Feb. 11. Conjunctivitis. Leucocytes 12,550, of which 7,781 were polymorphonuclears and 4,769 mononuclears. Temperature 39.6°C. Feb. 12. Conjunctivitis persists. Leucocytes 11,450, of which 7,786 were polymorphonuclears and 3,664 mononuclears. Temperature 39.8°C.

The development within 24 hours of the conjunctivitis, the rise in temperature with depression of the leucocytes and mononuclears, which endured for 48 hours, were indications for killing the animal. Hence on Feb. 12 the animal was killed.⁹

Autopsy.—The lung condition was such as has been described as a typical effect of the inoculation.

Aerobic Cultures.—Cultures on the usual media remained sterile.¹⁰

⁸ Usually more than one rabbit was inoculated at the same time, but in this instance only the course of the rabbit used for further transmission experiments is described.

⁹ In all experiments the animal was killed by a sharp blow, which dislocated the upper cervical vertebræ. In this way the complicating effects of ether anesthesia on the respiratory tract were avoided. The blow should be properly directed; otherwise the chest may be struck, thus causing contusion of the lung, or the skull may be broken with consequent profuse hemorrhages and aspiration of the blood, in which event the pathological picture is obscured.

¹⁰ As a routine practice aerobic blood cultures were made before the animal was killed. Also pieces of lung tissue were planted in 1 per cent dextrose broth, and in this medium plus rabbit blood. Anaerobic cultures of lung tissue will be described in another communication.

Second Passage. Rabbit B.—Average normal leucocyte count before inoculation 10,775, of which 5,604 were mononuclears. Feb. 12, 1919. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit A. Feb. 13. Leucocytes 7,600, of which 3,268 were mononuclears. Feb. 14. Killed.

Autopsy.—The lung appearance was typical.

Aerobic Cultures.—Remained sterile.

Third Passage. Rabbit C.—Average normal leucocyte count before inoculation 15,060, of which 7,461 were mononuclears. Feb. 14, 1919. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit B. Feb. 15. Leucocytes 5,675, of which 2,043 were mononuclears. Feb. 16. Leucocytes 8,760, of which 3,942 were mononuclears. Killed.

Autopsy.—Typical lung lesions, but to a milder degree.

Aerobic Cultures.—No growth of ordinary bacteria obtained.

A suspension of the lung tissue from this rabbit failed, however, to produce a similar effect in the succeeding rabbit. At this point the series of transmissions was terminated.

PROTOCOL 2.

Patient 16.—N. L., adult female. Mar. 28, 1919. Onset sudden with chills and fever. Mar. 29. Temperature 39°C.; muscular pains in back; prostration and weakness; epistaxis; unproductive cough; coryza and photophobia. Flushed face; injected conjunctivæ; congested pharynx; lungs, a few râles anteriorly and posteriorly on left side only. No other organs affected. Leucocytes 7,300; mononuclears 2,117. Cultivation of sputum and nasopharyngeal washings yielded *Pneumococcus* Type IV. Sputum injected into mouse yielded no Pfeiffer bacilli, only the pneumococci. On the 2nd day of illness the temperature declined, and the general condition improved. On the 3rd day a relapse occurred, but recovery began on the 4th day.

Animal Inoculation.—The unfiltered nasopharyngeal washings were obtained 36 hours after the onset and were inoculated into the lungs of Rabbit A.

First Passage. Rabbit A.—Mar. 29, 1919. Injected intratracheally with 3 cc. of the nasopharyngeal washings from Case 16. Mar. 30. Conjunctivitis. Leucocytes decreased from the norm of 8,200 to 6,700, and mononuclears from 4,100 to 2,680. Killed.

Autopsy.—Lungs showed the typical lesions.

Aerobic Cultures.—Free from growth.

Second to Sixth Passages. Rabbits B, C, D, E, and F.—Each rabbit was injected similarly with a suspension of the lung tissue of the immediately preceding rabbit of the series. All showed uniform clinical effects. After 24 to 48 hours there developed conjunctivitis, leucopenia, mononuclear depression, and varying temperature reactions. None died, but all were killed 48 hours after the injection, except Rabbit C which was killed after 24 hours.

Autopsy.—There were present varying degrees of the typical lung lesions. The lung lesions of the last (sixth) rabbit passage are shown in Figs. 1, 3, and 4.

Aerobic Cultures.—In each instance free from growth.

Further transmissions were not made and hence this series terminated in the sixth rabbit passage.

The two series of experiments recorded in Protocols 1 and 2 indicate that definite and consistent clinical and pathological effects were induced in two series of rabbits with materials derived from the nasopharynx of recent acute cases of influenza which were independent of the presence of ordinary aerobic bacteria.

The next series of three protocols is given in order to bring out the fact that the clinical and pathological effects regarded as typical appear even when ordinary bacteria are cultivable, and also that these bacteria are suppressible through successive inoculation while the typical effects continue to occur.

PROTOCOL 3.

Patient 6.—K., adult female. Jan. 27, 1919. Onset sudden with chills and fever. Jan. 28. Pains in the muscles of back and legs; prostrated and weak; severe frontal headache; mild unproductive cough. Temperature 38°C. Physical examination showed no signs in chest; conjunctivæ injected. Cultivation of sputum and nasopharyngeal washings yielded Pfeiffer bacilli and Pneumococcus Type IV. Symptoms persisted for 3 days, followed by an uneventful recovery.

Animal Inoculation.—The unfiltered nasopharyngeal washings obtained 24 hours after the onset of the symptoms were inoculated into the trachea of Rabbit A.

First Passage. Rabbit A (Text-Fig. 1, a).—Jan. 28, 1919. Injected intratracheally with 3 cc. of the nasopharyngeal washings from Case 6. Blood count of rabbit prior to inoculation showed 15,025 leucocytes, of which 8,414 were mononuclears. Jan. 29. Purulent conjunctivitis. Leucocytes 6,600, of which 2,244 were mononuclears. Jan. 30. Leucocytes 8,025, of which 2,006 were mononuclears. Killed.

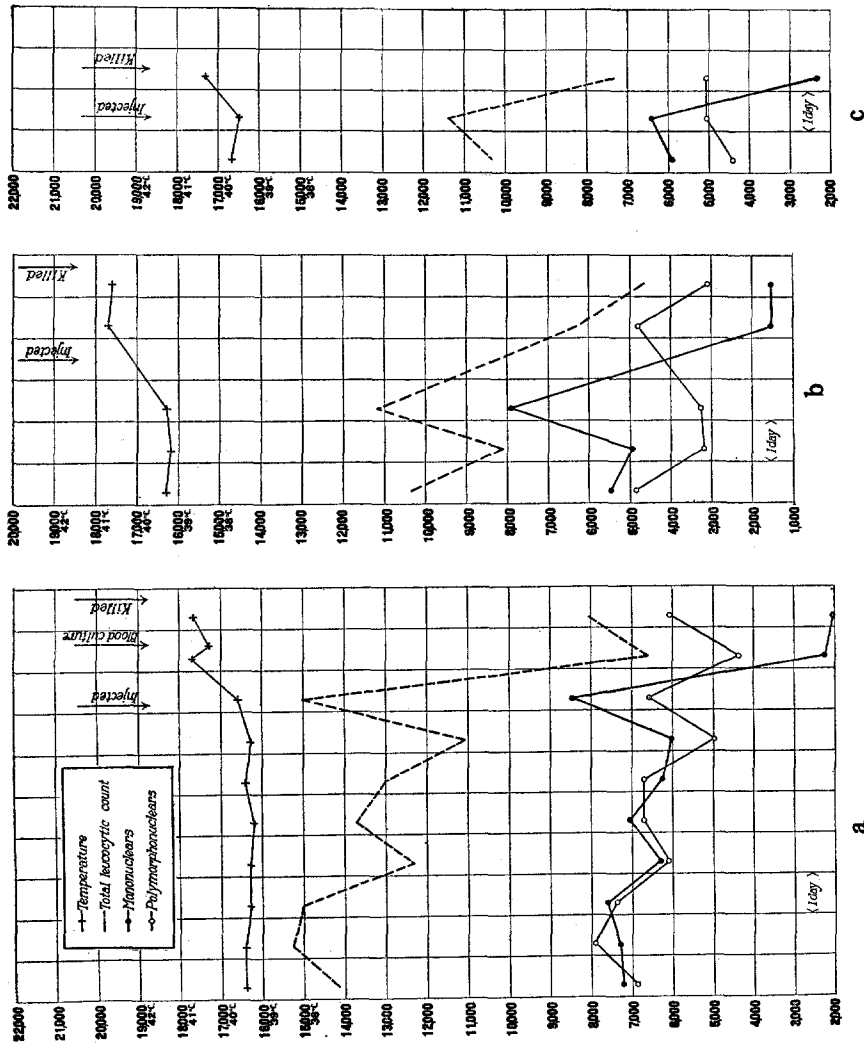
Autopsy.—Left lung showed gray hepatization with fibrinous pleuritis (lobar pneumonia); right lung, aside from a small area of atelectasis in upper lobe, showed edema and emphysema with large numbers of petechial hemorrhages.

Aerobic Cultures.—Left lung yielded a growth of Pneumococcus Type IV, avirulent for mice;¹¹ right lung yielded no growth of ordinary bacteria.

The right lung was employed for inoculation into the next rabbit.

Second Passage. Rabbit B (Text-Fig. 1, b).—Jan. 30, 1919. Injected intratracheally with 3 cc. of the suspension of tissue from right lung of Rabbit A.

¹¹ The significance of the ordinary bacteria encountered in these experiments will be dealt with in another communication.



TEXT-FIG. 1. *a*, *b*, and *c*. Effect on the blood count and temperature. The rise in temperature and the depression in the total white blood cell count caused by a deficiency of mononuclears are shown. (*a*) First rabbit passage of the nasopharyngeal washings from a case in the early stage of uncomplicated influenza (Patient 6). (*b*) Second rabbit passage from the same case. (*c*) Seventh rabbit passage from Patient 11.

Feb. 1. Killed after having shown a depression of 6,765 in the total count and 6,269 in the mononuclear count on the 2nd day after injection.

Autopsy.—Both lungs were edematous and emphysematous and showed multiple punctate hemorrhages.

Aerobic Cultures.—No growth obtained from heart's blood or lung tissue.

Third Passage. Rabbit C.—Feb. 1, 1919. Normal leucocyte count before inoculation was 10,950, of which 5,585 were mononuclears. Temperature 39.1°C. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit B. Feb. 2. Both conjunctivæ injected. Leucocytes 9,300, of which 3,255 were mononuclears. Temperature 39.7°C. Feb. 3. Conjunctivitis. Leucocytes 7,350, of which 2,850 were mononuclears. Temperature 40.5°C. Killed.

Autopsy.—Lungs showed typical hemorrhagic edema and emphysema.

Aerobic Cultures.—No growth.

Fourth Passage. Rabbit D.—Feb. 3, 1919. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit C. On the next 2 days the temperature rose from 39.3° to 39.5° and 40°C. Conjunctivitis was present with a mononuclear depression of 2,455 cells on the 1st day and 1,829 on the 2nd. Feb. 5. Killed.

Autopsy.—Besides a few areas of consolidation at the base measuring 5 mm. in diameter, the lungs showed edema, patches of emphysema, and multiple punctate hemorrhages.

Aerobic Cultures.—Heart's blood, no growth; lung tissue, lower lobes *Pneumococcus* Type IV, upper lobes no growth.

The upper lobes were employed in the next transmission experiment.

Fifth Passage. Rabbit E.—Feb. 5, 1919. Injected intratracheally with 3 cc. of a suspension of tissue from the upper lobes of lungs of Rabbit D. During the next 2 days the temperature rose from 39.5° to 40.4° and 39.8°C. Leucopenia was noted, with a mononuclear depression from 5,377 to 2,030 on the 1st day and 3,468 on the 2nd. Feb. 7. Killed.

Autopsy.—Lungs showed the typical lesions similar to those of Rabbit B.

Aerobic Cultures.—No growth.

Sixth Passage. Rabbit F.—The effects were an exact repetition of those of Rabbit E so that it is unnecessary to give them in detail.

Aerobic Cultures.—No growth.

Seventh Passage. Rabbit G.—Feb. 9, 1919. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit F. On the 2 following days the temperature rose from 39.6° to 39.8°C. Leucopenia developed, the count diminishing from 15,640 to 12,075 and 10,725 respectively, with a mononuclear depression from 8,289 to 2,415 and 3,325 cells. Conjunctivitis. Feb. 11. Killed.

Autopsy.—Lungs showed typical lesions similar to those of Rabbits B, C, E, and F.

Aerobic Cultures.—No growth.

Eighth Passage. Rabbit H.—Feb. 11, 1919. Temperature 39.7°C. Leucocytes 12,560, of which 5,275 were mononuclears. Injected intratracheally with

3 cc. of the suspension of lung tissue from Rabbit G. Feb. 12. Leucocytes 3,250, of which 2,015 were mononuclears. Temperature 40.35°C. Feb. 13. Leucocytes 4,300, of which 2,193 were mononuclears. Temperature 40.6°C. Died.

Autopsy.—Right lung showed consolidation and fibrinous pleuritis; left lung revealed typical hemorrhagic edema with emphysema.

Aerobic Cultures.—Left lung, no growth; right lung, *Micrococcus catarrhalis*.

The left lung was employed in the next transmission experiment.

Ninth Passage. Rabbit I.—Feb. 13, 1919. Leucocytes 11,200, of which 5,488 were mononuclears. Temperature 39.2°C. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit H. On the next 2 days the temperature rose to 39.6° and 39.8°C., the leucocytes diminished to 8,900 and 8,675 cells, and the mononuclears to 3,382 and 2,776 respectively. Feb. 15. Killed.

Autopsy.—Lungs showed the typical lesions.

Aerobic Cultures.—No growth.

Tenth Passage. Rabbit J.—Feb. 15, 1919. Injected with lung tissue from Rabbit I with similar results.

Eleventh Passage. Rabbit K.—Feb. 17, 1919. Injected similarly with lung tissue from Rabbit J. For 3 days the total leucocyte count was diminished from 10,120 to 8,320, 7,125, and 7,975, and the mononuclear count from 4,250 to 2,573, 2,494, and 1,674 respectively. Allowed to recover for experiment on immunity.

This series, therefore, was voluntarily terminated in the eleventh rabbit passage. It seems certain that it could have been continued for some time and possibly indefinitely. Besides what were regarded as typical results, this series included pneumonic infections with *Pneumococcus* Type IV and *Micrococcus catarrhalis* in the first, fourth, and eighth passages. Fortunately the pneumonic areas were restricted and did not lead to infection of both lungs. Hence the successive inoculation of the bacteria-free lung tissue could be continued. The death of Rabbit H of the eighth passage was the first to occur in our experiments.

PROTOCOL 4.

Patient 11.—R. N., adult male. Feb. 18, 1919. Onset sudden with chills and fever. Feb. 19. Temperature 39°C.; pulse 80. Prostration; general malaise; coryza and photophobia; unproductive cough. Physical examination showed conjunctivitis and congested pharynx; right lung, no signs; left lung, at base, few indefinite râles. Leucocytes 5,040, of which 1,764 were mononuclears. Cultures from sputum and nasopharyngeal washings yielded only *Streptococcus viridans*. Symptoms endured for 3 days, followed by an uneventful recovery.

Animal Inoculation.—The unfiltered nasopharyngeal washings obtained 24 hours after the onset of the symptoms were inoculated into the lungs of Rabbit A.

First Passage. Rabbit A.—Feb. 19, 1919. Leucocytes 13,960, of which 8,794 were mononuclears. Temperature 39.3°C. Injected intratracheally with 3 cc. of the nasopharyngeal washings from Case 11. Feb. 20. No change. Feb. 21. Conjunctivitis. Temperature 40.5°C. Leucocytes decreased to 6,100; mononuclears to 2,989. Killed.

Autopsy.—Lungs showed the typical lesions.

Aerobic Cultures.—No growth.

Second Passage. Rabbit B.—Feb. 21, 1919. Leucocytes 12,650, of which 6,451 were mononuclears. Temperature 39.7°C. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit A. On the next 2 days there were fever (41.3° and 40.6°C.), leucopenia (leucocytes 5,760 and 5,775), and mononuclear depression to 2,592 and 1,098 cells. Feb. 23. Killed.

Autopsy.—Lungs showed the typical lesions.

Aerobic Cultures.—No growth.

Third to Eighth Passages. Rabbits C, D, E, F, G, and H.—These rabbits developed, 24 hours after the intratracheal injection of the suspensions of lung tissue from the immediately preceding rabbit in the series, a pronounced leucopenia (in one case a decrease in leucocytes from 15,900 to 2,350), mononuclear depression (in Rabbit D, for example, from 7,632 to 940 cells), conjunctivitis, and fever (usually above 40.2°C.). The rabbits of the third, fourth, and fifth passages died 48 hours after injection; those of the sixth, seventh (Text-fig. 1, c), and eighth were killed in the usual manner.

Autopsy.—In all instances definite areas of consolidation were found in one or more lobes, while elsewhere the lung contained the typical areas of hemorrhage and edema accompanied with emphysema.

Aerobic Cultures.—The consolidated foci of the third, fourth, and fifth passages yielded *Pneumococcus* Type IV, and of the sixth, seventh, and eighth an atypical Type II *pneumococcus*.

Ninth Passage. Rabbit I.—Mar. 5, 1919. Leucocytes 14,225, of which 7,397 were mononuclears. Temperature 39.5°C. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit H. After 48 hours the temperature rose to 40°C., the leucocytes diminished to 10,225, of which 6,237 were mononuclears. This persisted for 2 days. Mar. 9. Killed.

Autopsy.—Lungs showed the typical lesions.

Aerobic Cultures.—No growth.

Tenth Passage. Rabbit J.—Average leucocyte count prior to inoculation was 15,353, of which 7,587 were mononuclears. Temperature 39.6°C. Mar. 9, 1919. Injected intratracheally with 3 cc. of the suspension of lung tissue from Rabbit I. After 24 hours, and for the next 3 days, the temperature rose to 40°C., the leucocytes diminished to 11,400, reaching 8,800 on the 3rd day, and the mononuclears decreased to an average of 4,886 cells. Mar. 13. Killed.

Autopsy.—Lungs showed the typical lesions.

Aerobic Cultures.—No growth.

With this, the tenth rabbit passage, the series was discontinued.

This series closely reproduces the previous one. The transmission experiments were discontinued with the tenth passage and at a time when the typical effects were still being produced regularly. Several instances of fatal intercurrent infection were encountered, the pneumonia present being associated with *Pneumococcus* Type IV or atypical Type II. But other portions of the lungs, which were free of these and other ordinary bacteria, were suitable for the transmission experiments.

PROTOCOL 5.

Patient 17.—S. C., adult male. Apr. 10, 1919. Sudden onset at night with symptoms of languor, chills, headache, and dull pain in the back. Apr. 11. General malaise; prostration; fever; headache; no cough or rhinitis. Physical examination showed conjunctivæ injected, pharynx congested, and lungs clear (also by radiographic examination). Other organs unaffected. Leucocytes 5,875, of which 2,059 were mononuclears. Blood culture sterile. Sputum not obtainable. A throat culture yielded no Pfeiffer bacilli on oleate agar (Avery medium¹²), but a Gram-negative coccus on blood agar plates. Nasopharyngeal washings yielded this coccus and *Streptococcus viridans*. On the 3rd day of illness the temperature was normal, and an uneventful recovery followed.

Animal Inoculation.—The unfiltered nasopharyngeal washings obtained 12 hours after the onset of symptoms were inoculated intratracheally into Rabbit A.

First to Third Passages. Rabbits A, B, and C.—These animals were employed for the first, second, and third passages respectively, the same methods being used as those described in the previous transmission experiments. Rabbit A died over night, Rabbit C after 2 days, and Rabbit B was killed. In all instances fever (above 40°C.) developed, with conjunctivitis and a prompt and marked leucopenia and mononuclear depression similar to those already given.

Autopsy.—The lungs revealed typical hemorrhagic edema, the hemorrhages varying from diffusely scattered small foci to large areas involving almost an entire lobe, sometimes of infarct shape. There was no definite consolidation.

Aerobic Cultures.—The lungs of the three animals yielded Type IV pneumococci.

*Fourth to Fifteenth Passages.*¹³—These were initiated with a filtrate, free from aerobic bacteria, of the suspension of lung tissue from Rabbit C.

In this experiment the pneumococcus present in the nasopharyngeal secretions was not suppressed during the first to third rabbit passages.

¹² Avery, O. T., *J. Am. Med. Assn.*, 1918, lxxi, 2050.

¹³ These experiments will be described in detail in a later communication.

But the effects in the animal, even though severe and fatal, bore so close a resemblance to those arising in the four previous series of rabbits that it was deemed advisable to free the material from the pneumococcus by filtration. The filtrate thus secured, free from aerobic organisms, was passed through a series of twelve animals in which the typical clinical and pathological effects were obtained.

Thus far we have dealt with cases of influenza which occurred during the epidemic of 1918-19. The next series of experiments relates to cases arising in the winter of 1920 of which three uncomplicated cases in the early stage of the affection were available for study.

PROTOCOL 6.

Patient 24.—H. J., adult female. Jan. 20, 1920. Onset sudden with chills and fever. Jan. 21. Prostration; photophobia; muscular pains; unproductive cough. Conjunctivitis and injected pharynx; lungs negative. Leucocytes 2,800, of which 336 were mononuclear. Cultures from the nasopharyngeal washings yielded numerous colonies of *Staphylococcus albus* and *Micrococcus flavus*, and a moderate number of Pfeiffer bacilli.

Animal Inoculation.—The filtered and unfiltered washings obtained 20 hours after the onset were inoculated into the lungs of three rabbits which, within 24 hours, developed conjunctivitis, fever, leucopenia, and mononuclear depression similar to those in the other instances cited. The animals were killed 48 hours after inoculation.

Autopsy.—The lungs showed the typical hemorrhagic, emphysematous, and edematous condition.

With the lungs of these rabbits another series of transmissions, consisting of three rabbits, two for the filtered material, to be described in another communication, and one for the unfiltered, was carried out. The results in each case were typical.

PROTOCOL 7.

Patient 26.—M. O., adult female. Feb. 4, 1920. Onset sudden with chills and fever. Temperature 40.1°C. Feb. 5. Fever; muscular pains; unproductive cough. Conjunctivitis; lungs clear. Leucocytes 6,000, of which 1,380 were mononuclears. Cultures from the nasopharyngeal washings yielded mainly *Staphylococcus albus* and also pneumococci, hemolytic streptococci, and occasional Pfeiffer bacilli.

Animal Inoculation.—The filtered and unfiltered nasopharyngeal washings obtained 30 hours after the onset were injected intratracheally into Rabbits A, B, and C, the last two receiving the filtrates. These animals showed the typical clinical and pathological effects, similar to those of the rabbits described in Protocols 1 and 2. No ordinary bacteria were detected in cultures or films of the lungs. The rabbits were killed 48 hours after the inoculation.

With the lungs of these animals another series of transmissions was carried out, with results which were regarded as typical. The gross appearance of the lungs of one rabbit is shown in Fig. 2. The lungs of one rabbit of the first passage of the filtered material (Rabbit C) were preserved in sterile 50 per cent glycerol for 4 months, and the lungs of Rabbit D, the second passage of the unfiltered washings, for 10 days. With these glycerolated lungs further transmissions were carried out to be described in another communication, until the experiment was voluntarily discontinued with the sixth rabbit passage. All showed the typical clinical and pathological effects, and in no instance were ordinary bacteria detected in cultures or in stained films from the lungs.

PROTOCOL 8.

Patient 27.—P. S., adult male. Feb. 9, 1920. Onset sudden with chills and fever. Feb. 10. Fever; muscular pains; sore throat; tracheal cough; headache; prostration. Lungs clear. Leucocytes 13,000, of which 10,140 were polymorphonuclears. Cultivation of nasopharyngeal washings yielded an almost pure culture of hemolytic streptococci and a few colonies of Pfeiffer bacilli. The question was raised of a complicating streptococcus sore throat in this patient.

Animal Inoculation.—The filtered and unfiltered nasopharyngeal washings obtained 20 hours after onset were injected into the lungs of five rabbits. The filtrates produced no effect; the unfiltered material caused abscess of the lung, from which hemolytic streptococci were isolated. The material was not inoculated further.

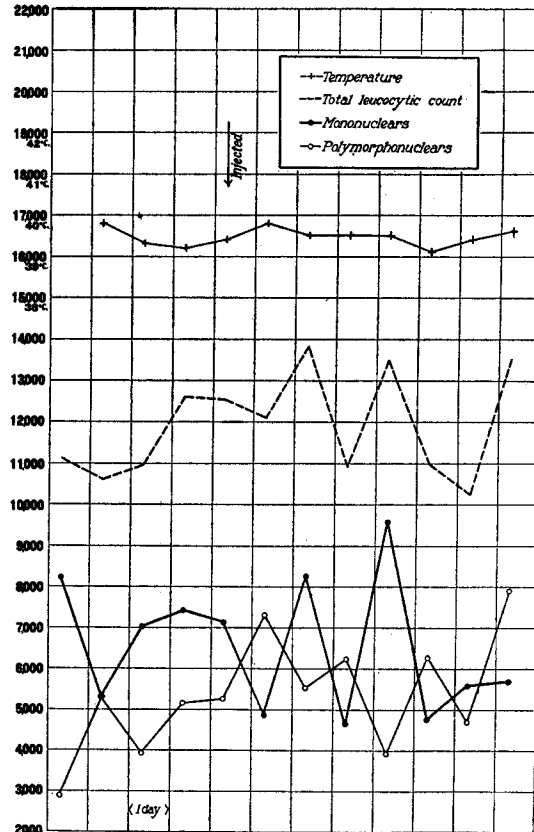
It is seen that the nasopharyngeal secretions of two of three cases of influenza during the second or 1920 epidemic gave rise in rabbits to clinical and pathological effects, independently of the presence of ordinary bacteria, similar to those obtained during the first or 1918 epidemic.

Negative Transmission Experiments.

In addition to the positive transmission experiments presented under Protocols 1 to 8, several cases of influenza, at somewhat later stages in the evolution of the disease, were studied in the same manner. The cases from the 1918 epidemic included five which were in the 3rd day of the disease when the washings were secured, four in the afebrile and convalescent stage, and two in course of a secondary pneumonia. From the 1920 epidemic one case only, on the 1st afebrile day, was studied.

The washings from the nasopharynx of these individuals were collected and injected into rabbits in the manner already described, and

in none of them was the characteristic effect on the blood count observed (Text-fig. 2). When a blood change did occur it was of the nature of a polymorphonuclear leucocytosis. The lung lesions, when any were present, consisted of lobar consolidation in which pneumococci, as a rule, were demonstrated.

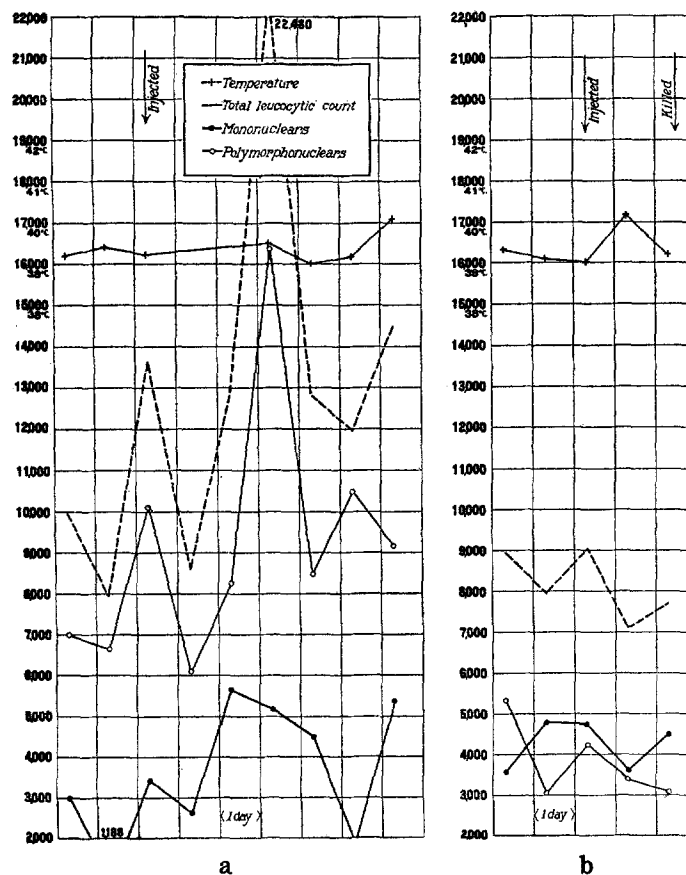


TEXT-FIG. 2. First rabbit passage of the nasopharyngeal washings from Patient 5. The washings were obtained at the beginning of the 3rd day of uncomplicated influenza. No effect on blood count and temperature.

Control Experiments (Text-Fig. 3, a and b).

The control tests consisted of the injection into the lungs of rabbits of saline solution, suspensions of normal rabbit lungs, normal rabbit serum, foreign protein, such as human ascitic fluid, bacteria of the

ordinary species,¹⁴ including Pfeiffer's bacillus and its poison as prepared by Parker's method,¹⁵ and finally, the nasopharyngeal secretions from fourteen persons free from influenza and tested in the epidemic



TEXT-FIG. 3, *a* and *b*. Effect on the blood count and temperature of intratracheal inoculations of control materials. (*a*) Inoculation of nasopharyngeal secretions from a normal individual free from an influenzal attack. A transient polymorphonucleosis on the 3rd day after inoculation is shown. (*b*) Inoculation of a suspension of normal rabbit lung. No effect on blood count and temperature.

¹⁴ Control experiments with ordinary bacteria injected intratracheally will be described in another communication.

¹⁵ Parker, J. T., *J. Immunol.*, 1919, iv, 331.

and interepidemic periods. Of the latter, seven suffered from early or later stages of coryza. The lung tissue of the inoculated rabbits was in turn reinoculated into two successive series of rabbits. None of the 55 animals inoculated with the control materials or these secretions showed the familiar clinical and pathological action; a few gave a polymorphonucleosis with frank lobar pneumonia, others a mononucleosis without lung involvement, and still others inconstant effects.

DISCUSSION.

The object of the present investigation was to determine, if possible, whether the secretions of the nasopharynx of individuals suffering from epidemic influenza exhibited on inoculation into animals any peculiarities of action or properties which would serve to distinguish them from the secretions of individuals not so affected. Obviously, the first requisite was a standard by means of which this action, or effect, could be detected. We sought one which was subject to measurement with at least a fair degree of accuracy and did not necessitate killing the inoculated animal. This criterion was found in the blood, associated with changes in the absolute and differential white blood count and correlated with the leucocytic curve in uncomplicated cases of epidemic influenza in man. A second criterion, observable after the animal had been killed, was discovered in certain hemorrhagic, edematous, and emphysematous changes in the lungs.

The next point was the determination of the relation of the changes noted in the leucocyte count and the lung structures to the ordinary bacterial flora of the nasopharynx. The fact that the changes were regularly observable, under favorable conditions, in the complete absence of ordinary bacteria in culture and in film, was regarded as particularly significant.

The deduction arrived at, therefore, as a result of the experiments carried out in rabbits was to the effect that patients with epidemic influenza, within at least the early stage of the obvious infection, carry in their nasopharyngeal secretions a substance which is not ordinary bacteria or their metabolic products. This substance when inoculated intratracheally into rabbits readily causes fever, leucocytic and particularly mononuclear cell depression, lung hemorrhage, edema, and em-

physema. Whatever this active substance is, it seems to disappear from or to diminish in the nasopharyngeal secretions of cases of epidemic influenza so as to be no longer discoverable by inoculation tests about 36 hours after the obvious symptoms of the disease have appeared, and to be absent from healthy persons and in other pathological conditions.

When implanted in the lungs of the rabbit this substance appears to increase, since it remains active through a long series of inoculations of the lung tissue in successive passages through rabbits.

The substance is readily filterable through Berkefeld filters. It is capable of surviving and apparently of multiplying in association with ordinary bacteria not only in the nasopharyngeal secretions in man but also in the lungs of rabbits, in the latter at least for a time.

When the unfiltered washings containing nasopharyngeal secretions of patients in early stages of epidemic influenza are injected intratracheally into rabbits, this substance when present exerts its peculiar action while some multiplication of ordinary bacteria (usually *Pneumococcus* Type IV) is going on in the lung. The successive passage of unfiltered emulsions of selected parts of the lungs, away from obviously infected and consolidated areas, leads often to rapid and complete disappearance of the ordinary bacteria and survival and possibly increase of this active substance.

No attempt will be made in this paper to define further the nature of the active substance or to relate it more accurately and specifically with the etiology of epidemic influenza.

SUMMARY.

An active substance has been detected, by the methods described, in five patients in early stages of epidemic influenza during 1918-19 and two patients in early stages of epidemic influenza during 1920. It was not detected in twelve cases of the same disease in which the onset of obvious symptoms occurred more than 36 hours before washing of the nasopharynx was carried out, nor was it found in the secretions of fourteen individuals free from the syndrome of influenza either during the epidemics or the interval between them.

With this substance a clinical and pathological condition has been induced in rabbits, affecting the blood and pulmonary structures

mainly, which could be maintained and carried through at least fifteen successive animals. For this reason, and also because of the dilution between passages, we are led to believe that we were dealing with the actual transmission of a multiplying agent rather than with a passive transference of an original active substance.

In some of the experiments secondary infections by ordinary bacteria were encountered. The relation of these microorganisms to this active substance will be dealt with fully in another communication. However, the essential effects were produced by a substance wholly unrelated to these bacteria.

The similarity that exists between the effects produced in rabbits on the blood and the lungs and those occurring in man in epidemic influenza provides a basis for further investigation on the inciting agent of epidemic influenza.

EXPLANATION OF PLATES.

PLATE 5.

FIG. 1. Gross lesions of a lung from Rabbit F, representing the sixth rabbit passage of the nasopharyngeal secretions from Patient 16. The hemorrhages, edema, and emphysema of the lung, more marked in the upper lobe, and absence of pneumonic consolidation are noteworthy. Natural size.

FIG. 2. Gross lesions of the lungs from Rabbit D, representing the second rabbit passage of the nasopharyngeal secretions from Patient 26. This case occurred in the second epidemic of 1920 and is to be compared with Fig. 1 derived from the first epidemic of 1918-19. Hemorrhages, edema, and emphysema are shown. A small area of atelectasis is seen at the inner margin of the lower right lobe. Natural size.

PLATE 6.

FIG. 3. Microscopic appearance of a section of the lung shown in Fig. 1. Edema and emphysema are present. A vessel is shown distended with blood, and mononuclears may be seen in the interalveolar tissues. \times about 190.

PLATE 7.

FIG. 4. A different section of the same lung. The small discrete hemorrhages, the edema, and the cellular exudate are shown. \times about 190.



M. L. Hedge
1913.

FIG. 1.



M. L. Hedge
1913.

FIG. 2.

(Olitsky and Gates: Nasopharyngeal secretions from influenza. I.)

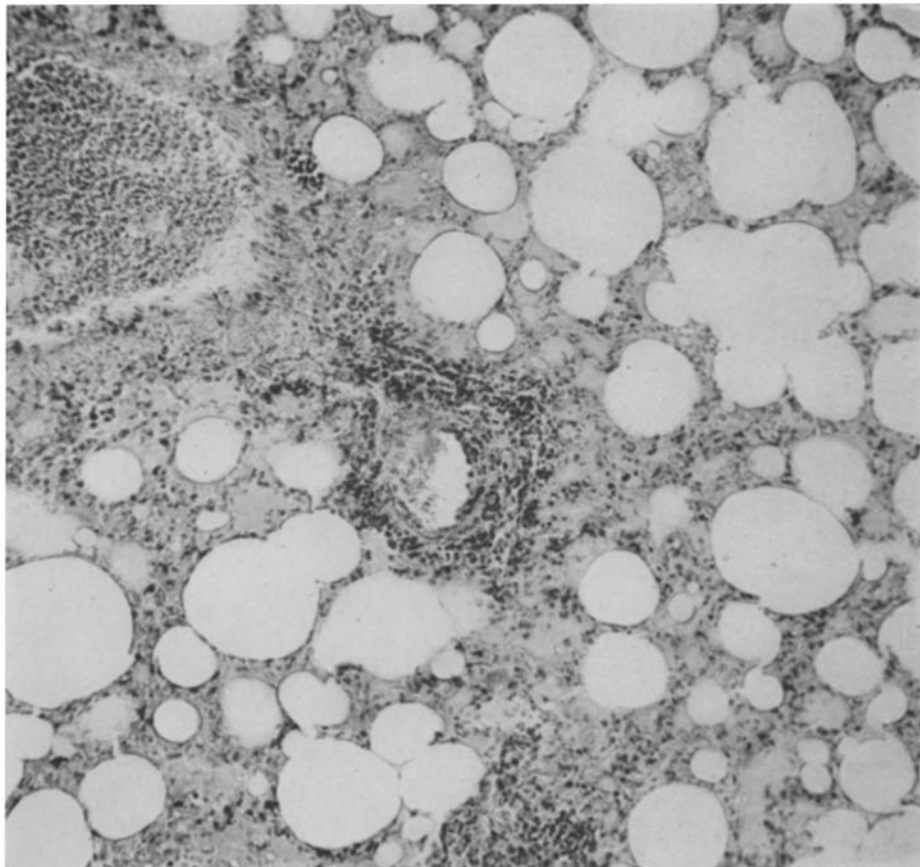


FIG. 3.

(Olitsky and Gates: Nasopharyngeal secretions from influenza. I.)

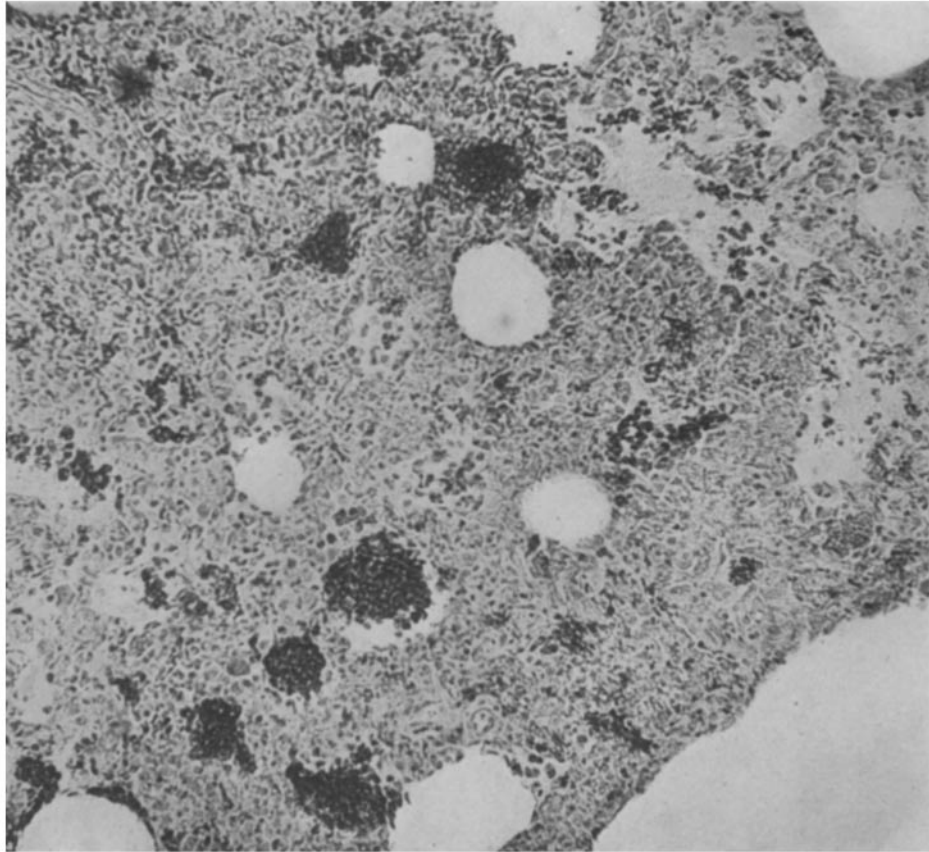


FIG. 4.

(Olitsky and Gates: Nasopharyngeal secretions from influenza. I.)